AD_____

Award Number: DAMD17-02-1-0458

TITLE: Integration of Pathologic Findings with Clinical-Radiologic Tumor Measurements to Quantify Response to Neoadjuvant Chemotherapy

PRINCIPAL INVESTIGATOR: William F. Symmans, M.D.

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: June 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Annual (1 Jun 2003 - 31 May 2004)

4. TITLE AND SUBTITLE
Integration of Pathologic Findings with Clinical-Radiologic Tumor Measurements to Quantify Response to Neoadjuvant Chemotherapy

5. FUNDING NUMBERS
DAMD17-02-1-0458

6. AUTHOR(S)

William F. Symmans, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Texas
M.D. Anderson Cancer Center
Houston, TX 77030

E-Mail: fsymmans@mdanderson.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The aim of the project is to develop and test a new method to quantify the proportion (percent) of cancer that is residual after neoadjuvant chemotherapy using standard radiologic and/or clinical measures of tumor size that are integrated with pathologic information about the amount of cancer within each tumor. We have determined that tumor cellularity significantly decreases as a result of neoadjuvant (pre-operative) chemotherapy compared to control untreated breast cancers managed by surgery alone. However, the extent and variability of reduction of cellularity is considerable, particularly in the tumors that partly respond, and this shifts the distribution of residual tumor burden closer to complete response in those cases. Overall, this distribution indicates that many breast cancers are more responsive to neoadjuvant chemotherapy than measurement of tumor diameter alone would indicate. Therefore, size alone is not a sufficient measure of the tumor response to treatment. We have combined our measure of cancer cellularity with the radiological tumor measurements with the gross and microscopic pathologic changes in the breast and axillary lymph nodes after chemotherapy to determine a measure of relative breast cancer response. This Residual Cancer Index closely correlates with T-stage and appears to organize the responses into a meaningful distribution that allows a detailed view of nonresponsive tumors and can then be used to conduct rank order statistical analyses without dividing the distribution of responses into arbitrary categories. Using this approach we have determined that low proliferation (Ki-67 < 15%), bcl-2 overexpression, and tau overexpression significantly predict greater resistance to neoadjuvant chemotherapy with paclitaxel, 5-FU, doxorubicin, and cyclophosphamide.

14. SUBJECT TERMS
breast cancer, chemotherapy, neoadjuvant, response, pathologic

15. NUMBER OF PAGES
22

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION:
A more accurate way to measure breast cancer response to treatment would improve the rate of yield of information from clinical trials of neoadjuvant chemotherapy. It would also provide a more useful standard with which to compare the relevance of pathologic findings in residual cancer and with which to test those molecular biomarkers that show promise to predict response to treatment. We are developing and testing a method to quantify tumor response, using clinical, radiologic, and pathologic information that is applicable to most clinical practices. We are comparing the molecular evidence of cell survival and proliferative activity in the residual cancer cells and pathologic changes in the residual carcinoma from neoadjuvant chemotherapy as they relate to the amount of tumor response.

BODY:
Task 1. To determine the best measurement of tumor size after treatment (Months 1 - 24)

a. *Review of mammography and ultrasound imaging studies from before and after treatment, estimate average of 10 cases per month. (Months 1 - 24)*
b. *Two radiologists to independently make measurements and document the preferred imaging modality for each tumor. (Months 1 - 24)*
c. *Obtain the clinical tumor measurements and the categorical assessments of tumor response from the clinical trial database. (Months 1 - 6)*
d. *Pathology review of slides, reports, and specimen radiographs to document residual tumor size and other histopathologic findings for subsequent tasks. (Months 1 - 24)*
e. Complete the statistical analyses. (Months 24 - 25)

The Department of Defense approved the IRB for human subjects research on December 22, 2002. In year one we have identified a cohort of 108 patients who received neoadjuvant chemotherapy for breast cancer and reviewed their pathology materials (see task 2). Pathological data included: tumor size, % invasive cancer, % in situ cancer, % cancer cellularity within the tumor, and cytomorphologic changes within residual cancer cells.

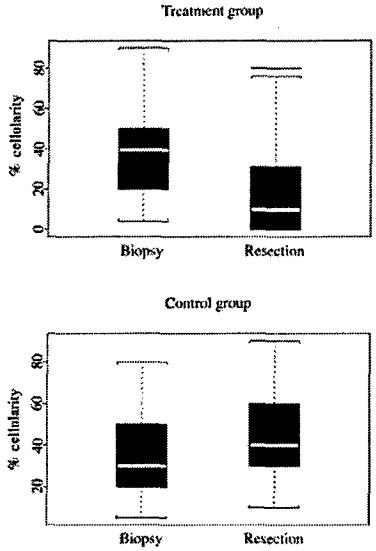
In year two we completed our analysis of the pathological changes in % cancer cellularity before and after neoadjuvant chemotherapy, compared this to the clinical response and pathologic T-stage after treatment. We presented the findings as a poster at the San Antonio Breast cancer Symposium in December, and published these results as a paper in CANCER in March, 2004. In year two we completed the radiological review of all radiological materials from 85 of these patients (the review of remaining patients' material is ongoing) and combined these results with the pathological data to determine an index score for the proportion of residual cancer burden after chemotherapy relative to the cancer burden before treatment began.

Task 2. Calculation of percent residual cancer volume (Months 1 - 27)

a. *Immunohistochemical staining of tumor sections for cytokeratins. (Months 1 - 24)*
b. *Image analysis to calculate percent cancer cellularity by area. (Months 3 - 24)*
c. *Calculation of tumor volume using the best measure of tumor size - see task 1. (Months 24 - 26)*
d. *Calculation of percent residual cancer volume and statistical analyses. (Months 25 - 27)*

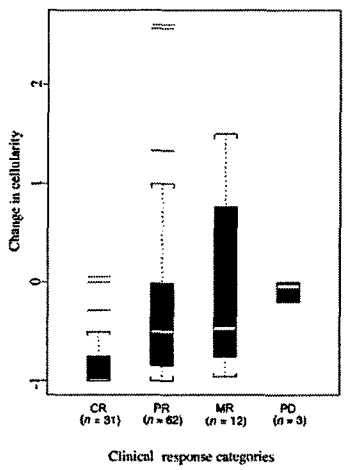
In year one the cancer cellularity within the tumor area was measured from hematoxylin and eosin stained tumor sections for the pre-treatment diagnostic core biopsy and the post-treatment

resection specimen from 108 breast cancers from women who were treated with pre-operative (neoadjuvant) chemotherapy in the clinical trial (ID98-240). These were compared to the cancer cellularity in the diagnostic core biopsy and the surgical resection specimen from a control group of 120 breast cancers that did not receive pre-operative chemotherapy. The findings were that the % cancer cellularity in an untreated resected breast cancer is slightly higher than in the corresponding diagnostic core biopsy sample obrtained before surgery (40%, versus 30%). However, after neoadjuvant chemotherapy the %cellularity in the residual breast cancer is significantly less than in the pre-treatment core biopsy sample from that patient's tumor (10%, versus 40%). Therefore, neoadjuvant chemotherapy significantly reduces the % cellularity within the breast cancer.



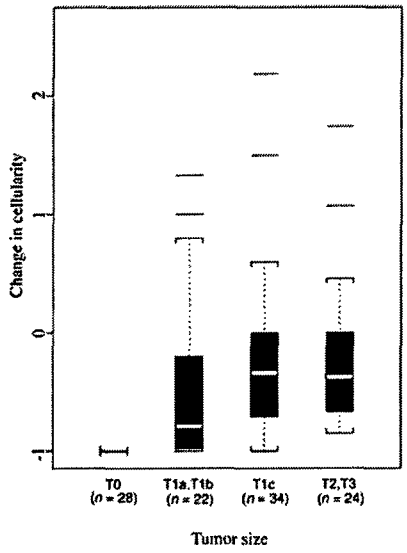
The tumor cellularity in treatment and control groups is summarized using a boxplot. The black rectangle in each case indicates the 25th and 75th percentiles of the distribution with median indicated by white horizontal lines within the rectangles. The figure indicates that there was a significant overall decrease in cellularity of resection specimens compared to biopsy specimens for the treatment group (Paired Wilcoxon signed rank test p-value <0.01), while a significant increasing trend was noted in the percentage of tumor cellularity in patients from the control group (Paired Wilcoxon signed rank test p-value < 0.01).

We calculated the relative change in tumor cellularity as follows: Relative change in tumor cellularity = (percentage of tumor cellularity at resection - percentage of tumor cellularity in biopsy) / percentage of tumor cellularity in biopsy. Medians (range) of the change in tumor cellularity in treatment and control groups were –0.67 (-1, 2.6) and 0 (-0.75, 5), respectively. We determined that this is highly variable in different patients' tumors and that it is most obvious in patients who achieve more marked clinical response and those with the least residual breast cancer after treatment. Reduction in cancer cellularity was not seen in those few patients with stable or progressive disease, and was highly variable in patients who achieved minimal or partial response.



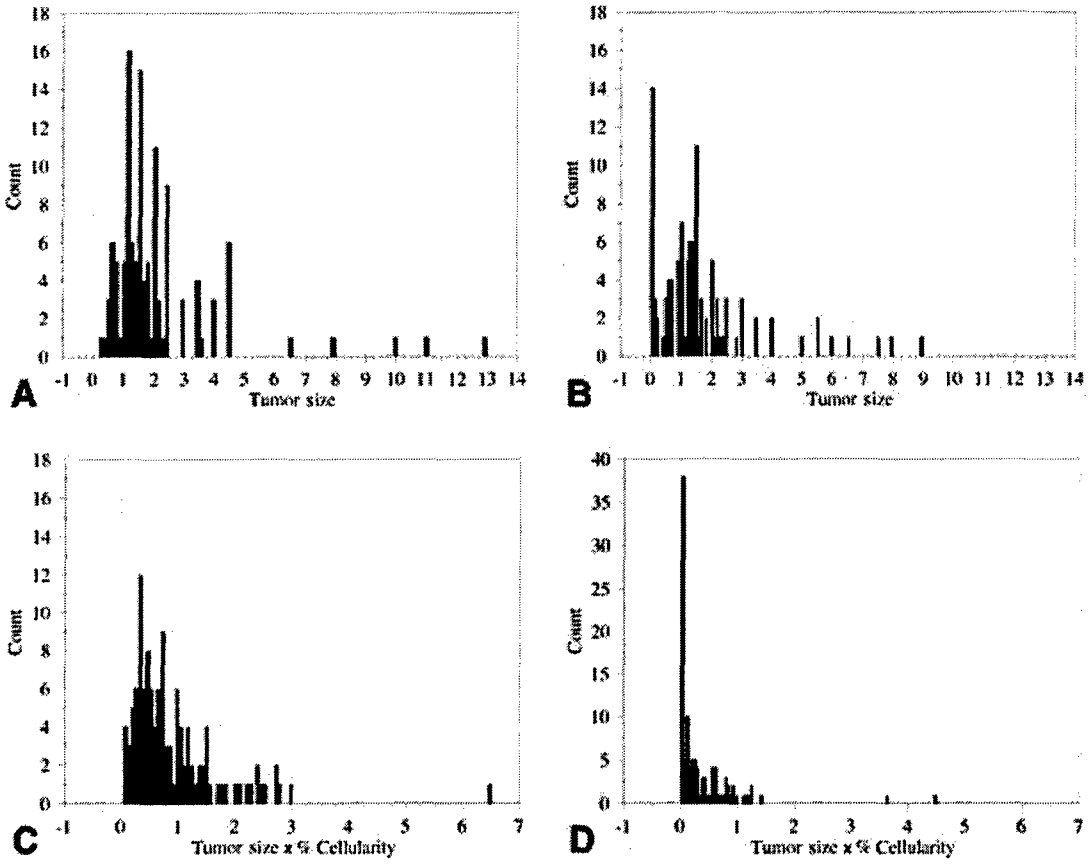
The relative change in tumor cellularity was computed with the following formula:
relative change in tumor cellularity (percentage tumor cellularity at resection percentage tumor cellularity in the core needle biopsy) / percentage tumor cellularity in the core needle biopsy. Negative values indicated lower cellularity at resection compared with the core needle biopsy specimen. A minimum value of 1.0 was equated with a pathological complete response in the breast primary. Patients who achieved clinical CR had significantly more reduction in tumor cellularity than other patients (Kruskal-Wallis test p-value <0.01, p-value is also <0.01 after combining minimal response and progressive disease).

Categorization by residual pathologic tumor status shows that changes in cellularity were highly variable for all residual tumor classifications (pT1–pT3), but that pT1a and pT1b tumors (combined) showed the greatest reduction in cellularity. A minority of tumors in each residual tumor classification had increased cellularity after treatment.



Relative changes in cellularity categorized by tumor stage show that T1a and T1b residual tumors demonstrate the greatest change in cellularity within residual breast cancer. However, changes in cellularity were found to be highly variable throughout T1, T2, and T3 residual tumors. Tumor size was categorized using the revised American Joint Committee on Cancer TNM staging system.

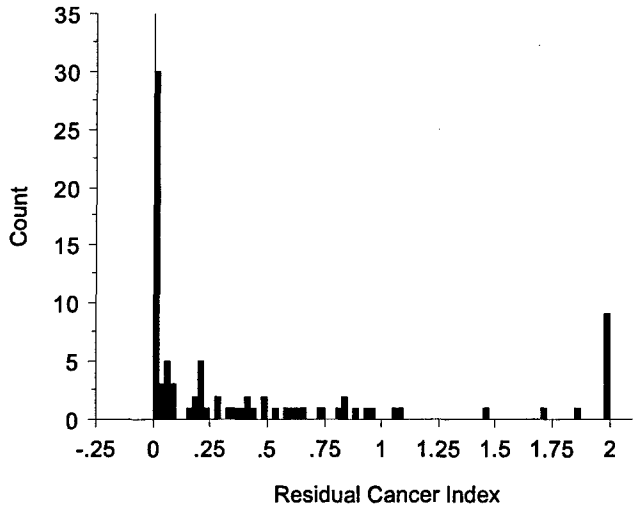
The combined product of tumor diameter from the resection specimen and the cancer cellularity within the resected tumor was compared with tumor diameter alone in the treated and control groups. The frequency distribution for tumor size alone is similar in tumors from patients who were treated with surgery alone (A) and from tumors from patients who were treated with neoadjuvant chemotherapy then surgery (B). The product of size and cellularity has a similar distribution in the tumors from patients who were treated with surgery alone (C), but is quite different in the tumors from patients who were treated with neoadjuvant chemotherapy then surgery (D). The shape of that distribution (D) suggests that the population of treated tumors all tend towards a zero product of size and cellularity (complete response) rather than having a skewed normal or bimodal distribution. This appears to be a meaningful pattern of distribution because it suggests that a majority of tumors respond to treatment to some extent. The implication of these graphs is that the incorporation of cancer cellularity (shown to relate to clinical response and pathological T-stage after treatment) as a variable in the overall measurement of tumor response is likely to be an improvement over using size alone.



The above analyses were published as an article in CANCER (see references). The combination of residual tumor size and cancer cellularity is an indicator of residual tumor burden, but does not include information about the size and pathological features of the tumor before treatment began.

In year two we have reviewed all the radiological materials from 85 of these patients and have defined a residual cancer index score that includes radiological and histopathological information about the tumor before treatment began as well as gross and histopathological information about the residual primary tumor site in the breast and the axillary nodal basin. A formula was designed to include these variables into a residual cancer index score:

RCI score = [(Residual Pathological tumor area x Proportion invasive cancer) + (# Positive lymph nodes x Diameter largest metastasis)] / (Pre-treatment Radiological tumor area x Proportion invasive cancer)



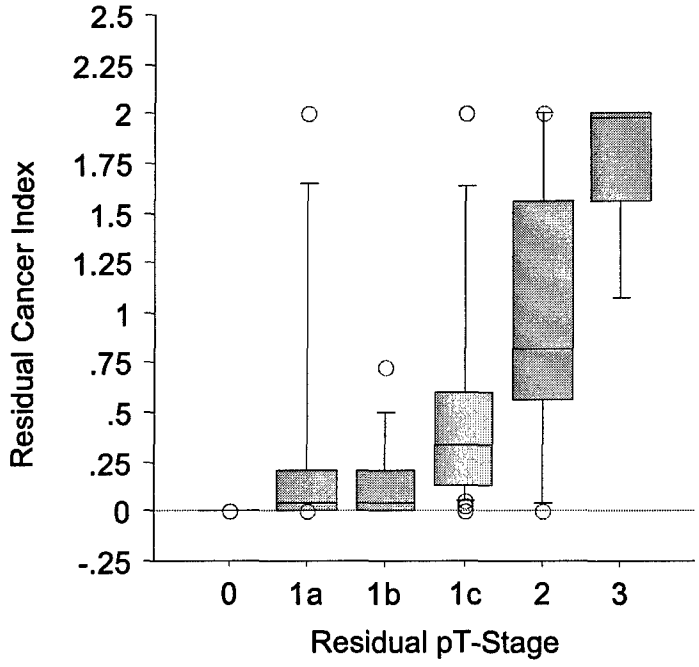
The distribution of the residual cancer index (above) is similar to the distribution for the combination of size x cancer cellularity. Values ≥ 2.0 were assigned a score of 2.0. Pathological complete response (pT0 N0) was achieved in 18 patients, but a total of 30 patients have a residual cancer index that is close to zero. This indicates a group of patients with residual disease but whose response is nearly complete. The distribution demonstrates a range of responses that could be used as a measure of response for statistical analyses. When the Residual Cancer Index score was compared to the p T-Stage of these tumors the result was highly significant (Kruskal-Wallis test, $p<0.0001$).

Kruskal-Wallis Rank Info for residual Cancer Index
Grouping Variable: pT Stage

	Count	Sum Ranks	Mean Rank
0	18	198.000	11.000
1a	7	276.500	39.500
1b	13	453.000	34.846
1c	29	1529.500	52.741
2	13	811.000	62.385
3	5	387.000	77.400

One case was omitted due to missing values.

It is encouraging that this Residual Cancer Index score is significantly related to T-Stage of the resudual tumor, because that is a robust indicator of long-term disease-free survival. However, it is also evident from the box plot below that there is variation of the Residual Cancer Index scores within each stage category and overlap across different categories. We suggest that the Residual Cancer Index score might better organize the response of some tumors in the study, such as those with sparse cellularity in a larger area, those with small T-stage but many positive nodes, and small node-positive tumors that were not much affected by treatment.



We have now begun to compare the Residual Cancer Index scores to pathological characteristics in the residual tumors (Task 3) and in the breast cancers before treatment began (Task 4).

Task 3. To assess the pathology of residual cancers and correlate these with tumor response. (Months 12 - 30)

a. *Immunohistochemical staining of residual tumor sections for Ki-67/MIB-1, HIF-1a, bcl-2, bcl-XL, and NF-kß. (Months 12 - 20)*
b. *TUNEL assay for apoptosis in residual tumor sections. (Months 20 - 24)*
c. *Microscopic interpretation of immunohistochemistry and TUNEL staining. (Months 20 - 28)*
d. *Complete the statistical analyses with tumor response. (Months 28 - 30)*

Task 3 is not yet complete, but an interim analysis of the immunohistochemical staining of residual cancer cells is presented for the available tissues from these patients. Immunohistoichemistry results were dichotomized as follows: Ki-67 ≥ 15% of nuclei defined as positive, bcl-2 cytoplasmic staining intensity ≥ 2+ (range 0 – 3) defined as positive, any bcl-6 nuclear staining defined as positive, any NF kappa B nuclear staining defined as positive, p53 ≥ 5% of nuclei defined as positive, any survivin staining defined as positive, tau cytoplasmic staining intensity ≥ 2+ (range 0 – 3) defined as positive.

		Residual pT-Stage	Residual Tumor Size x Cellularity	Residual Cancer Index
	n =	p value *	p value ^	p value ^
Ki-67	45	NS	0.01	NS
bcl-2	49	0.04	NS	NS
bcl-6	45	NS	NS	NS
NFkB	46	NS	NS	NS
p53	45	NS	NS	NS
Survivin	47	NS	NS	NS
Tau	44	NS	NS	NS

* Chi-Square test, ^ Mann-Whitney U test, NS is not significant (p>0.05)

There is not consistent or strong relationship between the expression of these biomarkers and residual tumor pT-Stage, residual tumor burden, or residual cancer index. Bcl-2 expression in the residual tumor cells was more common in the higher T-stages and greater proliferation (Ki-67 ≥ 15%) was more common when there was more residual tumor burden (size x cellularity).

Task 4. To test selected potential biomarkers for prediction of tumor response. (Months 24 - 34)

a. *Immunohistochemical staining of pre-treatment tumor samples for Ki-67/MIB-1 and p53. (Months 24 - 30)*
b. *Retrieval of results from Her-2/neu tests from pathology reports. (Months 24 - 27)*
c. *Microscopic interpretation of immunohistochemical staining and histopathologic biomarkers. (Months 28 - 32)*
d. *Complete the statistical analyses with tumor response. (Months 32 - 34)*

Task 4 is not yet complete, but an interim analysis of the immunohistochemical staining of residual cancer cells is presented for the available tissues from these patients. Immunohistochemistry results were dichotomized as follows: Ki-67 ≥ 15% of nuclei defined as positive, bcl-2 cytoplasmic staining intensity ≥ 2+ (range 0 – 3) defined as positive, p53 ≥ 5% of nuclei defined as positive, tau cytoplasmic staining intensity ≥ 2+ (range 0 – 3) defined as positive. Her-2/*neu* and ER results are being retrieved for all the patients but these have not yet been analyzed.

	n =	Residual pT-Stage	Residual Tumor Size x Cellularity	Residual Cancer Index
		p value *	p value ^	p value ^
Ki-67	52	0.04	0.05	0.03
bcl-2	52	0.0005	<0.0001	<0.0001
p53	48	NS	NS	NS
Tau	55	NS	NS	0.05

* Chi-Square test, ^ Mann-Whitney U test, NS is not significant (p>0.05)

Breast cancers with greater proliferation (Ki-67 ≥ 15%) were associated with smaller residual tumor pT-Stage, less residual tumor burden, and smaller Residual Cancer Index scores. This is a meaningful result because the tumor proliferation index before treatment has been previously shown to be related to greater probability of achieving a complete pathological response, versus residual disease. Breast cancers with bcl-2 overexpression had significantly greater residual tumor pT-Stage, residual tumor burden, and Residual Cancer Index scores. This is an interesting finding

because other studies have shown only borderline significance of bcl-2 overexpression to predict complete pathological response versus residual disease. Our analyses demonstrate that bcl-2 overexpression is probably more predictive of the amount of residual tumor burden and higher Residual Cancer Index scores. That makes sense when we consider the underlying hypothesis that bcl-2 overexpression would confer more resistance. It is interesting to note that overexpression of tau protein is associated with a higher Residual Cancer Index score. We identified from a different study using gene expression microarray experiments from pre-treatment FNA tumor samples in a different cohort of patients receiving T/FAC chemotherapy that elevated tau gene expression was strongly predictive of residual disease, versus complete pathological response. We were able to demonstrate in these patients that the immunohistochemical overexpression of tau was related to higher Residual Cancer Index scores (more residual cancer relative to the original tumor burden). In the final year of funding we aim to use immunohistochemistry to investigate the expression of some of the other molecules that we have identified from the gene expression studies.

Task 5. Compilation of patient follow-up from clinical trial database and statistical analyses for disease free interval and survival. (Months 30 - 36)

Work on Task 5 is scheduled to begin in year 3 of funding. The cohort of patients who are being studied received sequential chemotherapy with paclitaxel then 5-FU, doxorubicin, and cyclophosphamide (T/FAC) and currently have follow-up of 4 years. A separate population of 80 patients have been identified, all of whom received FAC chemotherapy, and follow-up of at least 8 years is available for all. The pathological and radiological materials are being retrieved for review and those results will used to calculate the Residual Cancer Index score for comparison with time to progression by the end of the funding period. It is hoped that this will determine whether the Residual Cancer Index as a measure of response has prognostic significance.

KEY RESEARCH ACCOMPLISHMENTS:

Key research accomplishments from this study to date are:

- Demonstration that cancer cellularity within the tumor is significantly decreased by neoadjuvant chemotherapy, and is most obvious and variable in the partial response and minimal response (stable disease) categories and, similarly, in tumors staged as T1 after treatment.
- Mathematical definition of a Residual Cancer Index score that incorporates radiological and histopathological information about the tumor before treatment and gross and histopathological information about the residual tumor and axillary nodes after the completion of neoadjuvant chemotherapy.
- Distribution of the residual tumor burden (tumor size x cellularity) and the Residual Cancer Index score demonstrate more clearly than the tumor size alone that many patients respond to neoadjuvant chemotherapy, some closely approximate complete pathological response, and the extent of residual cancer in those with residual disease is variable and can be represented as a distribution.
- These distributions of residual tumor burden and Residual Cancer Index are similar in shape and appear likely to be biologically meaningful and amenable to mathematical modeling.
- These distributions of residual tumor burden and Residual Cancer Index are related to the expression of known predictive biomarkers (Ki-67 and bcl-2) and a new biomarker (tau).

REPORTABLE OUTCOMES:

See Key Research Accomplishments above.

Abstract submitted to the San Antonio Breast Cancer Symposium for December, 2003.
Rajan R, Poniecka A, Smith T, Yang Y, Whitman G, Fiterman DJ, Pusztai L, Kuerer H, Hortobagyi GN, Symmans WF. Tumor cellularity of breast cancer as a variable in the pathological assessment of response following neoadjuvant chemotherapy.

Published Paper:
Rajan R*, Poniecka A*, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. ***Cancer*** 2004;100:1365-73.

The definition, distribution, and correlations of the Residual Cancer Index will be reportable when these studies are completed. Also, the biomarker studies of molecular characteristics in the residual tumors (task 3) and the pre-treatment tumor samples (task 4) will be reportable. Finally, the prognostic follow-up studies (task 5) will also be reportable.

CONCLUSIONS:

1. Assessment of cancer cellularity within the measured tumor bed provides meaningful information about tumor response following therapy.
2. Planned future studies of this variable with radiologic tumor measurements (before and after treatment) from this clinical trial are likely to yield valuable results.
3. Refinement of the assessment of cancer cellularity using cytokeratin immunohistochemical stains will be studied.

REFERENCES:

Abstracts
Rajan R, Poniecka A, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. San Antonio Breast Cancer Symposium, December 2003

Peer Reviewed Publications

Rajan R, Poniecka, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. ***Cancer*** 2004;100:1365-73.

APPENDIX:

Pdf file of publication:

Rajan R, Poniecka, Smith TL, Yang Y, Frye F, Pusztai L, Fiterman DJ, Gal-Gombos E, Whitman G, Rouzier R, Green M, Kuerer H, Buzdar AU, Hortobagyi GN, **Symmans WF**. Change in tumor cellularity of breast cancer following neoadjuvant chemotherapy as a variable in the pathological assessment of response. ***Cancer*** 2004;100:1365-73.

Change in Tumor Cellularity of Breast Carcinoma after Neoadjuvant Chemotherapy As a Variable in the Pathologic Assessment of Response

Radhika Rajan, M.B., Ch.B.[1]
Anna Poniecka, M.D.[2]
Terry L. Smith, M.S.[3]
Ying Yang, M.S.[3]
Deborah Frye, R.N.[4]
Lajos Pusztai, M.D., D.Phil.[4]
Derek J. Fiterman, B.S.[1]
Eva Gal-Gombos, M.D.[5]
Gary Whitman, M.D.[6]
Roman Rouzier, M.D.[4]
Marjorie Green, M.D.[4]
Henry Kuerer, M.D.[7]
Aman U. Buzdar, M.D.[4]
Gabriel N. Hortobagyi, M.D.[4]
W. Fraser Symmans, M.D.[1]

[1] Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[2] Department of Pathology, University of Miami, Miami, Florida.

[3] Department of Biostatistics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[4] Department of Breast Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[5] Department of Pathology, Mount Sinai Medical Center, Miami, Florida.

[6] Department of Radiology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[7] Department of Surgery, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Supported by the Department of Defense Breast Cancer Research Program (DAMD 17-00-1-0296) to W. Fraser Symmans.

Address for reprints: W. Fraser Symmans, M.D., Department of Pathology, Box 85, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009; Fax: (713) 745-5709; E-mail: fsymmans@mdanderson.org

Received November 25, 2003; accepted January 6, 2004.

DOI 10.1002/cncr.20134

BACKGROUND. Complete pathologic response of breast carcinoma to neoadjuvant chemotherapy is a well defined outcome that correlates with prolonged survival. Categorization of incomplete response depends on accurate measurement of residual tumor size but is complicated by the variable histopathologic changes that occur within the tumor bed. In the current study, the authors investigated the contribution of assessing tumor cellularity in the pathologic evaluation of response to chemotherapy.

METHODS. The slides from diagnostic core needle biopsy and the subsequent matched resection specimens were examined in 240 patients with breast carcinoma: 120 "treated" patients who received neoadjuvant chemotherapy and 120 "control" patients who received primary surgical management within a few weeks of diagnosis. Clinical response and residual tumor size were evaluated in 108 treated patients who completed a clinical trial with paclitaxel and then received combined 5-fluorouracil, doxorubicin, and cyclophosphamide chemotherapy. Tumor cellularity was assessed from hematoxylin and eosin-stained tissue sections as the percentage of tumor area that contained invasive carcinoma.

RESULTS. After neoadjuvant chemotherapy, tumor cellularity decreased from a median of 40% in core needle biopsy to 10% in resection specimens ($P < 0.01$; Wilcoxon signed rank test). The cellularity of core needle biopsy (median, 30%) tended to underestimate the cellularity of resection specimens (median, 40%) in the control group ($P < 0.01$). Changes in cellularity varied within each clinical response category, particularly partial response and minor response. The greatest reduction was observed in the cellularity of residual primary tumors that measured ≤ 1 cm (pathologic T1a [pT1a] and pT1b tumors), but changes in cellularity varied in the pT1, pT2, and pT3 residual tumor categories. The shape of the distribution of tumor size, expressed as the greatest dimension in cm, was similar in the control group and the treatment group (excluding complete pathologic response); however, when residual tumor size and cellularity were combined, the distribution of pathologic response shifted left (toward complete response) with a steep decline, suggesting that many tumors had a large reduction in cellularity but little change in the tumor size.

CONCLUSIONS. Cellularity of the tumor mass was reduced significantly by neoadjuvant chemotherapy, and the change varied widely in different categories of clinical response. Although residual tumors measuring ≤ 1 cm in greatest dimension had the most reduction in tumor cellularity, there was broad variability for all residual tumor groups (pT1–pT3). The frequency distribution of residual tumor size was altered markedly by the inclusion of tumor cellularity, indicating that the product of pathologic size and tumor cellularity may provide more accurate pathologic response information than tumor size alone. ***Cancer* 2004;100:1365–73.**

KEYWORDS: tumor cellularity, neoadjuvant chemotherapy, clinical response, tumor size.

The response of primary breast carcinoma to neoadjuvant chemotherapy correlates with survival. Patients who achieve a complete pathologic response are reported to have significantly improved disease free and overall survival.[1–5] Patients with smaller primary tumors are more likely to achieve a complete pathologic response.[6] The frequency of complete pathologic response (3–30%) depends on the clinical tumor classification and the type of chemotherapy used.[1,2,7] However, 60–80% of patients achieve partial or minor responses, and their prognosis is variable; therefore, further refinement of response assessment would be informative for this predominant group.[6,8–12]

Histologic evidence of response to preoperative chemotherapy was investigated previously in bone pathology, in which it was found that the percent tumor necrosis was the most significant prognostic factor in patients with osteosarcoma.[13] Recently, it was demonstrated that categories of histologic change independently were predictive of 5-year survival in patients with breast carcinoma after multimodality therapy.[14] We hypothesize that measurement of tumor cellularity, defined as the percentage of invasive tumor comprised of tumor cells, represents a potentially informative histologic measure of the differential response of primary breast tumors to chemotherapy. The objective of this study was to determine whether there

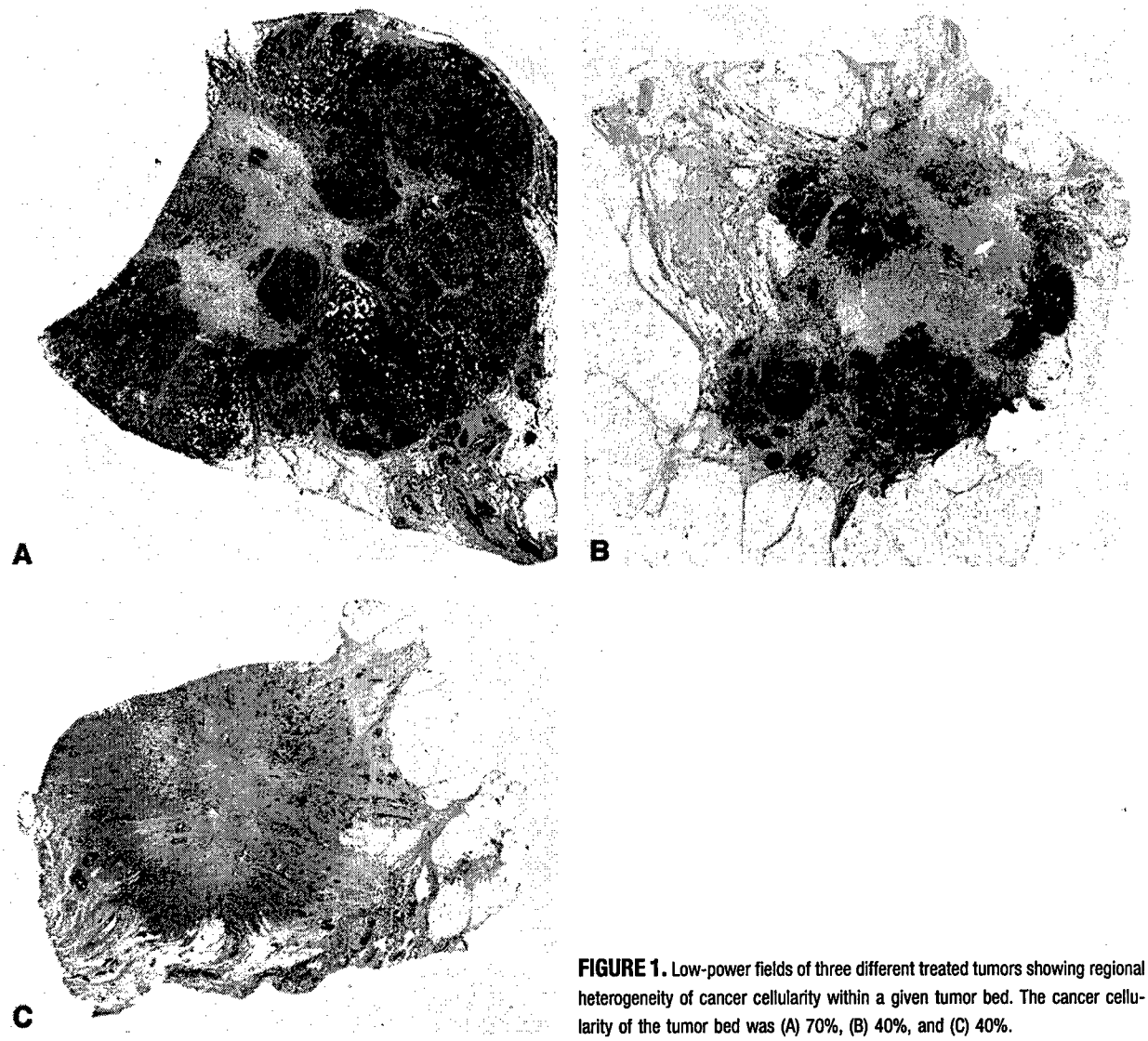


FIGURE 1. Low-power fields of three different treated tumors showing regional heterogeneity of cancer cellularity within a given tumor bed. The cancer cellularity of the tumor bed was (A) 70%, (B) 40%, and (C) 40%.

TABLE 1
Statistics of Cellularity (%) and Pathologic Tumor Size

Group	Cellularity (%)		Tumor size (cm)
	Core biopsy	Resected specimens	
Control group (n = 120 patients)			
Median	30	40	1.5
Range	75 (5–80)	80 (10–90)	12.8 (0.2–13.0)
Mean ± SD	38 ± 20	44 ± 20	2.1 ± 1.9
Treatment group (n = 120 patients)			
Median	40	10	1.3
Range	80 (10–9)	80 (0–8)	9.0 (0.0–9.0)
Mean ± SD	42 ± 21	18 ± 21	1.7 ± 1.7

SD: standard deviation.

are changes in tumor cellularity after chemotherapy, to ascertain whether there is variation in the extent of change in the different clinical response categories and residual tumor classifications, and what (if any) impact the inclusion of tumor cellularity may have on the distribution of pathologic tumor size.

MATERIALS AND METHODS

Patient Population

The patient population consisted of 240 patients with invasive breast carcinoma. The treated group was comprised of 120 patients who received neoadjuvant chemotherapy at the University of Texas M.D. Anderson Cancer Center between December 1998 and April 2001. Most treated patients (108 of 120 patients) were entered onto a clinical trial (ID 98-240) and were randomized to receive either weekly paclitaxel (150 mg/m^2 over 16 weeks for lymph node positive disease and 80 mg/m^2 over 12 weeks for lymph node negative disease) or paclitaxel given at 21-day intervals (225 mg/m^2) for 4 cycles. After completion of paclitaxel, all patients received 4 additional cycles of 5-fluorouracil (500 mg/m^2), doxorubicin (50 mg/m^2), and cyclophosphamide (500 mg/m^2) (T/FAC) before surgery. The control group was comprised of 120 patients who were treated by primary surgical management up to 4 weeks after core needle biopsy. All patients underwent core needle biopsy (14-gauge or 18-gauge) of the tumor for initial diagnosis followed by surgical resection, either as primary management (control group) or after neoadjuvant chemotherapy (treated group). Inclusion in this study required the availability of hematoxylin and eosin-stained histologic sections both from the initial core needle biopsy and from the subsequent resection specimen. Pathologic review and data analysis were conducted in accordance with an Institutional Review Board protocol that was approved by The University of Texas M. D. Anderson Cancer Center (LAB02-010).

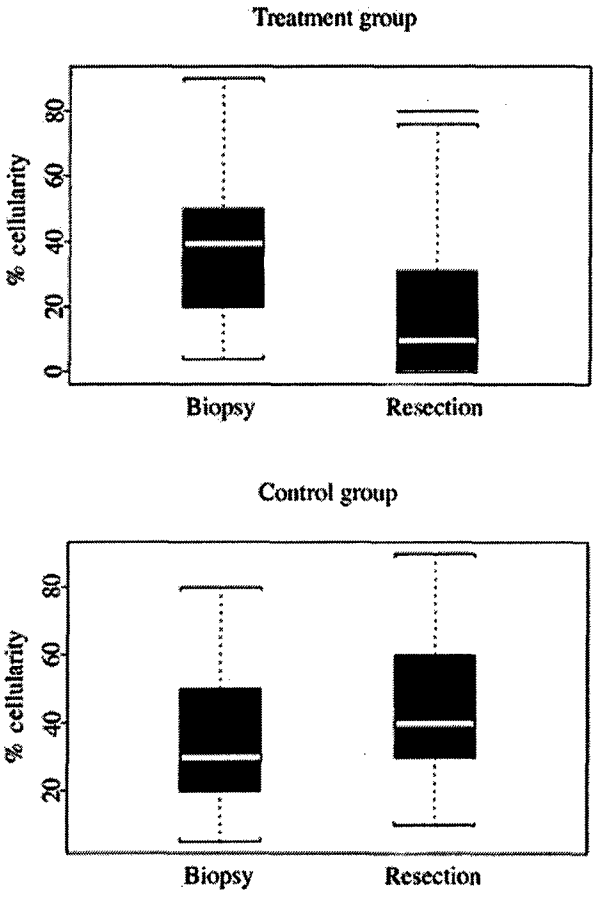


FIGURE 2. A box plot of the tumor cellularity in the core needle biopsies and resection specimens from the control group and the treatment group shows that a significant decrease in cellularity occured within the treatment group ($P < 0.01$). The colored rectangle indicates the 25th and 75th percentiles of each distribution with the median indicated by the white horizontal line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles, and single black lines represent individual patients outside of this range.

Assessment of Cellularity Within the Tumor

Sections of the tumor cross-sectional area were reconstructed from 1) mapping the tissue section code from the report to the macroscopic description of the tumor bed, 2) known macroscopic tumor dimensions from the report, and 3) comparison with available specimen radiographs. The boundaries of the tumor area were then outlined on the slides with ink. Computer-generated images of known areas were created in 10% increments to simulate different microscopic patterns of cancer and were used for initial visual training.

TABLE 2
Categorization of the Change in Tumor Cellularity, Tumor Size (cm), and Tumor Size Multiplied by Cellularity according to Clinical Response and Residual Tumor Stage[a]

		Change in tumor cellularity			Tumor size (cm)		Tumor size × cellularity		
Variable	No.	Median	Range	Mean ± SD	Median	Mean ± SD	Median	Range	Mean ± SD
Response									
CR	31	−1.0	0.95 (−1.0, −0.05)	−0.85 ± 0.3	0.0	0.8 ± 1.6	0.0	1.2 (0.0, 1.2)	0.09 ± 0.24
PR	62	−0.6	3.2 (−1.0, 2.2)	−0.3 ± 0.7	1.4	1.5 ± 1.4	0.2	4.5 (0.0, 4.5)	0.38 ± 0.62
MR	12	−0.5	2.48 (−0.98, 1.5)	−0.1 ± 0.9	2.1	2.7 ± 2.1	0.8	1.4 (0.0, 1.4)	0.7 ± 0.4
PD	3	−0.05	0.16 (−0.2, −0.04)	−0.1 ± 0.09	1.3	3.9 ± 4.4	0.98	3.35 (0.25, 3.6)	1.6 ± 1.8
Tumor stage									
T0	28	−1.0	0.0 (−1.0, −1.0)	−1.0 ± 0.0	0.0	0.0 ± 0.0	0.0	0.0 (0.0, 0.0)	0.0 ± 0.0
T1a	7	−0.6	1.9 (−0.98, 1.0)	−0.3 ± 0.8	0.4	0.3 ± 0.17	0.1	0.15 (0.001, 0.15)	0.08 ± 0.07
T1b	15	−0.8	2.3 (−0.98, 1.3)	−0.5 ± 0.7	0.9	0.8 ± 0.16	0.09	0.45 (0.009, 0.46)	0.1 ± 0.1
T1c	34	−0.3	3.1 (−0.97, 2.2)	−0.2 ± 0.7	1.5	1.5 ± 0.25	0.3	1.2 (0.0, 1.2)	0.4 ± 0.4
T2	17	−0.4	2.7 (−0.85, 1.9)	−0.07 ± 0.7	2.5	2.9 ± 0.8	0.5	1.3 (0.12, 1.4)	0.6 ± 0.4
T3	7	−0.7	0.9 (−0.85, 0.0)	−0.5 ± 0.4	6.5	6.9 ± 1.3	0.8	4.25 (0.25, 4.5)	1.6 ± 1.7

SD: standard deviation; CR: complete response; PR: partial response; MR: minor response; PD: progressive disease.
[a] Change in cellularity was defined as (% cellularity of resection − % cellularity of core needle biopsy) / % cellularity of core needle biopsy.

Cellularity within the tumor area was assessed from the slides by estimating the percentage area of the overall tumor bed that was comprised of invasive tumor cells. The complete cross-sectional area of the tumor bed was studied to account for the heterogeneous distribution of tumor cells within a given tumor bed (Fig. 1). Three pathologists independently reviewed the percentage tumor cellularity in the first 70 specimens, and there was nearly complete concordance between pathologists. One pathologist then completed the analysis. In specimens with multifocal disease, cellularity was assessed in the same tumor mass that had been sampled by core needle biopsy. Cellularity was recorded in 10% increments from 10% to 100%, with additional values of 1% and 5% for minimal cellularity. The proportion of invasive carcinoma was then calculated.

Clinical Response Categories

The assessment of clinical response was based on change in tumor size from pretreatment clinical measurements to posttreatment clinical and radiologic measurements. The clinical measurement was the product of the two greatest palpable perpendicular dimensions of the tumor. Clinical response was categorized into four groups: a complete response (CR) was defined as complete resolution of all tumor determined by physical examination and imaging studies; a partial response (PR) was defined as incomplete reduction > 50% in tumor size; a minor response (MR) was defined as a reduction in tumor size < 50%; and progressive disease (PD) was defined as an increase in tumor size. Pathologic size was defined as the greatest dimension of residual invasive tumor and was categorized using the revised American Joint Committee on Cancer TNM staging system.[15]

Statistical Analyses

Distributions of cellularity percentages among groups are summarized graphically using box plots. The shaded rectangles in the box plots delineate the 25th and 75th percentiles of each distribution, with the median indicated by a horizontal white line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles. Black lines then represent individual results outside of this range. The distributions of 1) residual pathologic tumor size and 2) the product of residual pathologic tumor size and tumor cellularity are summarized graphically by histograms. Measurements of cellularity in core needle biopsy and resection specimens were compared using the Wilcoxon signed rank test. All P values presented are two-sided, and P values < 0.05 were considered statistically significant. Statistical analyses were performed using SAS software (version 8.0) and Splus software (version 6.0). The relative change in tumor cellularity was computed with the following formula: relative change in tumor cellularity = (percentage tumor cellularity at resection − percentage tumor cellularity in the core needle biopsy) / percentage tumor cellularity in the core needle biopsy. Negative values indicated lower cellularity at resection compared with the core needle biopsy specimen. A minimum value of − 1.0 was equated with a CR.

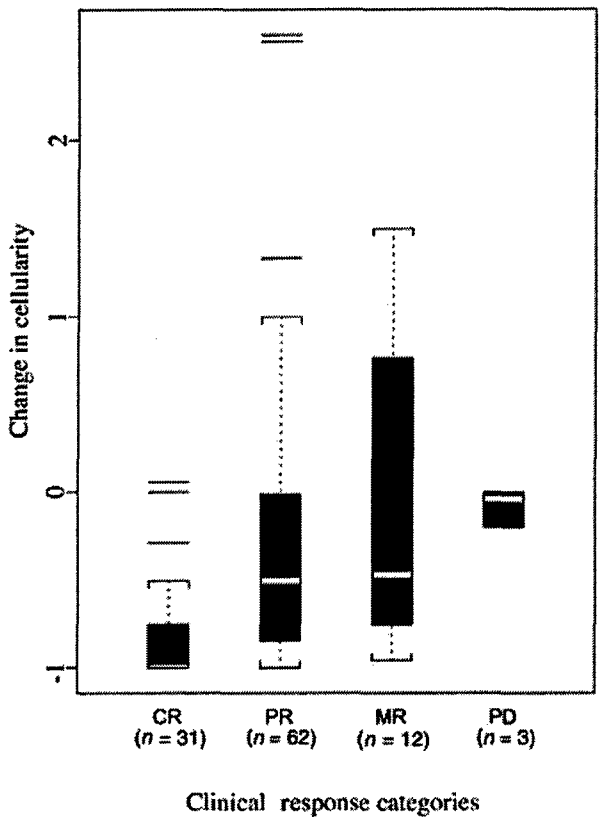


FIGURE 3. Relative change in tumor cellularity for each clinical response category: complete response (CR), partial response (PR), minor response (MR), and progressive disease (PD). Relative change in tumor cellularity was calculated as follows: (percentage tumor cellularity at resection − percentage tumor cellularity in core needle biopsy)/percentage tumor cellularity in core needle biopsy. The colored rectangle indicates the 25th and 75th percentiles of the distribution, and the median is indicated by the white horizontal line within the rectangle. The outer boundary brackets delineate the 2.5th and 97.5th percentiles, and short horizontal lines represent individual patients outside of this range.

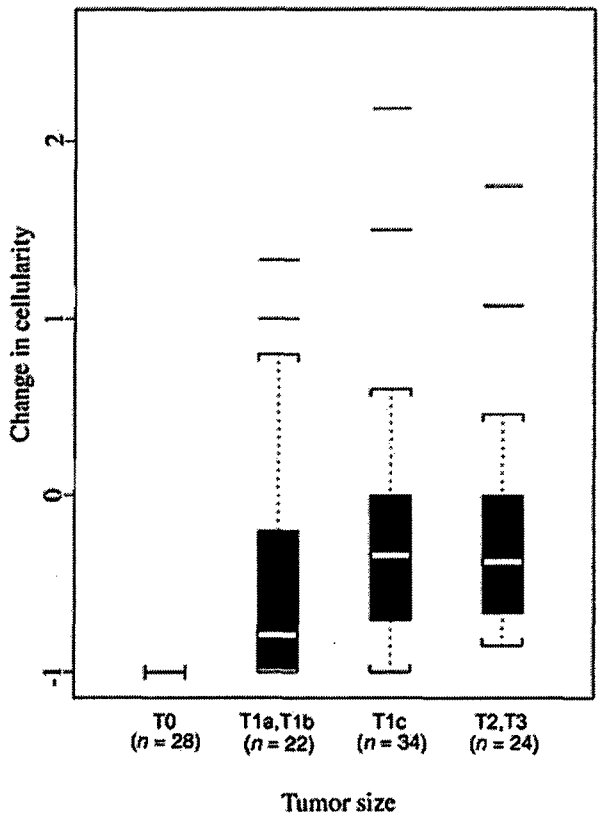


FIGURE 4. Relative changes in cellularity categorized by tumor stage show that T1a and T1b residual tumors demonstrate the greatest change in cellularity. However, changes in cellularity were found to be highly variable in T1, T2, and T3 residual tumors. Tumor size was categorized using the revised American Joint Committee on Cancer TNM staging system. This box plot format is the same as that used in Figure 3.

RESULTS

Statistics of the percentage tumor cellularities are presented for all groups (Table 1). Within the treated group, the median tumor cellularity decreased significantly from 40% in the core needle biopsies to 10% in the resected tumors ($P < 0.01$; Wilcoxon signed rank test). Tumor cellularity in patients from the control group increased from a median of 30% (core needle biopsy) to 40% (resected tumor; $P < 0.01$), indicating that core needle biopsy specimens may underestimate the overall cellularity at resection. These data are summarized in Figure 2 using a box plot.

Clinical response data were available for the 108 patients who received T/FAC neoadjuvant chemotherapy. The response rates in the current series (29% clinical CR, 57% PR, 11% stable disease, and 3% PD) are in agreement with those reported in most studies[1–12] and for this clinical trial.[7] The change in tumor cellularity relative to the starting value in the core needle biopsy was compared with clinical response and residual pathologic tumor (pT) status (Table 2). Relative changes in cellularity were highly variable in all four clinical response groups, particularly for patients who achieved a PR or an MR (Table 2, Fig. 3). Change in cellularity is related to clinical response: There was a median 50% reduction in tumor cellularity in the PR and MR groups (Table 2), although some tumors had increased cellularity, and the few tumors that progressed had no median change in tumor cellularity (Fig. 3). Categorization by residual pathologic tumor status shows that changes in cellularity were highly variable for all residual tumor classifications (pT1–pT3), but that pT1a and pT1b tumors (combined) showed the greatest reduction in cellularity

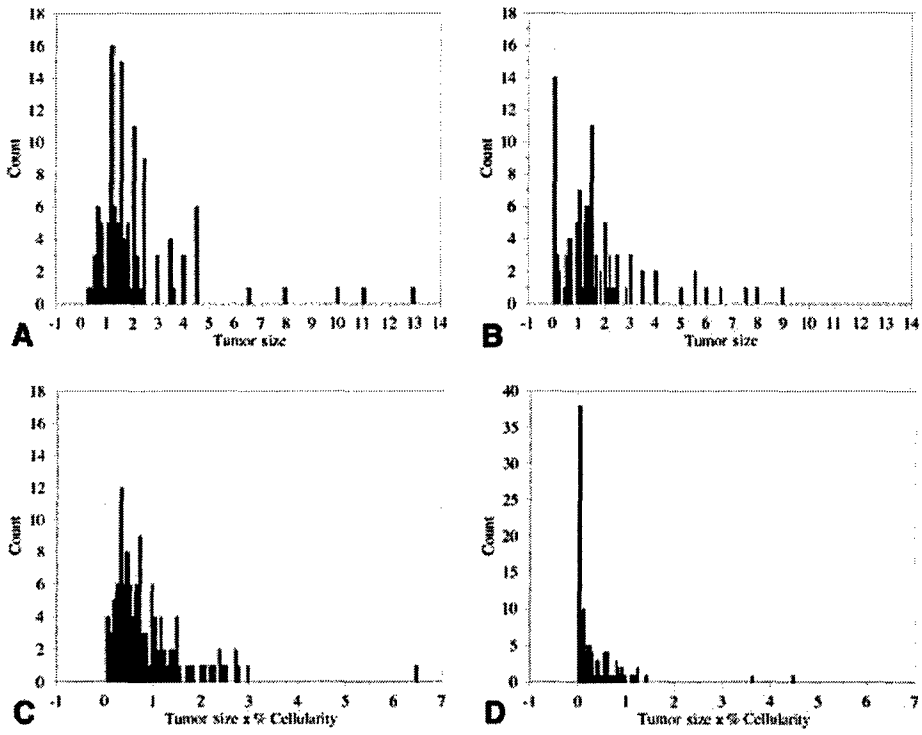


FIGURE 5. Histograms of pathologic tumor size (in cm) for the (A) control group and (B) treatment group show that the shapes of these distributions are similar (excluding the peak of complete pathologic responses in the treatment group). Histograms of the product of cancer cellularity and tumor size for the (C) control group and (D) treatment group show that the shape of the distribution for the control group is similar to that for tumor size (A). However, in treated tumors, the shape of the distribution changes to become a steeply inversely sloping curve. The control group is shown in red (A and C), and the treated group is shown in black (B and D).

(Fig. 4). Residual pT1a and pT1b tumors had similar median reductions in cellularity. A minority of tumors in each residual tumor classification had increased cellularity after treatment (see Fig. 4, positive values).

The frequency distributions of pathologic tumor size alone have similar shapes in the control group (Fig. 5A) and the treatment group when pathologic CRs are excluded in the treatment group (Fig. 5B). However, the product of pathologic size and tumor cellularity produces a steeply inversely sloped distribution in the treatment group (Fig. 5D) whereas the shape of the distribution in control group is similar to the distribution for size alone (Fig. 5C). The product of cellularity and size dramatically changes the distribution of residual tumor pathology in the treated group, causing a shift toward CR. This indicates that chemotherapy in some tumors can reduce cellularity dramatically but affects the overall size of the tumor only minimally. We propose that the product of residual size and cellularity may be a more clinically relevant measure of tumor response than assessing tumor size alone.

DISCUSSION

Clinical trials consistently indicate that the extent of response of primary breast carcinoma to neoadjuvant chemotherapy correlates with disease-free and overall survival.[1–12] The currently used categories of clinical response (namely, CR, PR, MR, and PD) are defined by the change in tumor size from pretreatment clinical and/or radiologic measurements to posttreatment clinical, radiologic, and pathologic measurements. However, residual tumor size is influenced by variable pathologic changes that occur within the tumor bed. Chemotherapy-induced fibrous stromal involution is reported to occur in up to 67% of tumors[16] and can result in clinical and macroscopic overestimation of residual tumor size. There is clearly a role for the development and validation of new histologic approaches to augment the pathologic and clinical assessment and to provide information concerning the differential response to neoadjuvant chemotherapy, particularly for tumors that achieve less than a pathologic CR.

The current study assessed the role that microscopic assessment of tumor cellularity may have in the pathologist's evaluation of tumor response. There is precedent for using microscopic assessments of the percentage tumor area or cellularity in breast pathology, such as in the assessment of the amount of intraductal component of tumor sections and in the assessment of estrogen receptor, progesterone receptor, Her-2/*neu*, and proliferation index (Ki-67) immunostaining.[17,18] In the field of bone pathology, it has been shown that histopathologic measurement of the percent tumor necrosis is the most significant prog-

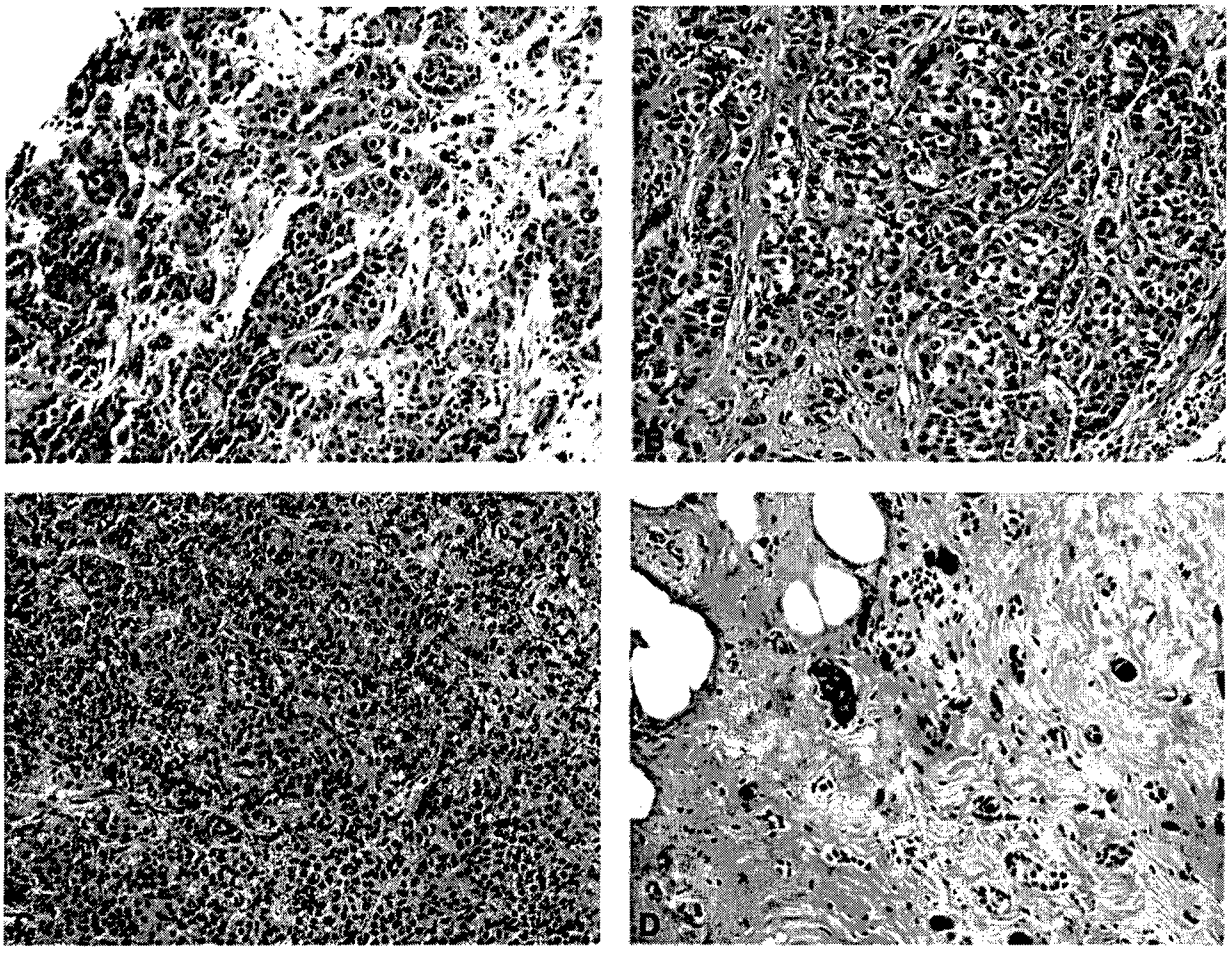


FIGURE 6. Two partially responding tumors with a similar decrease in tumor size but with markedly different changes in cellularity after neoadjuvant chemotherapy. (A, B) One tumor decreased from 2.0 cm to 1.8 cm and showed an increase in cellularity. A pretreatment core needle biopsy with a cellularity of 70% is shown in A, and a posttreatment tumor with a cellularity of 80% is shown in B. (C, D) The second tumor decreased from 1.7 cm to 1.5 cm and showed a decrease in cellularity. A pretreatment core needle biopsy with a cellularity of 90% is shown in C, and a posttreatment tumor with a cellularity of 5% is shown in D (original magnification × 10).

nostic factor in patients with osteosarcoma who are treated with preoperative chemotherapy.[13] We defined the size of the residual tumor bed; then, we estimated the overall cellularity of invasive tumor within that tumor bed. A potential benefit of this approach in the pathologic assessment after chemotherapy is that it bypasses the difficulties in measuring the greatest dimension of invasive tumor that is distributed unevenly within the residual tumor bed as scattered islands of residual disease.

The current results showed that the cellularity of the tumor mass is reduced significantly by neoadjuvant chemotherapy and that change is widely variable between individual patients and in the different categories of clinical response and residual tumor sizes. Relative change in cellularity varies widely in tumors that achieve a PR of MR and in the different residual tumor classifications. Figure 6 illustrates two partially responsive tumors that had similar decreases in tumor size after chemotherapy yet showed markedly different changes in cellularity. Changes in tumor size alone do not represent response entirely. Tumor cellularity in patients from the control group increased from a median of 30% to 40% ($P < 0.01$), indicating that core needle biopsy may underestimate the overall cellularity at resection. Preferential sampling by core needle biopsy of the fibrotic center in that subset of tumors may lower the median.[19] It is possible that artifactual tissue crushing from the automated core needle biopsy device may compress the cellular component more than the intervening stroma, hence slightly decreasing apparent cellularity. There may be differ-

ences in cell areas from different fixation and processing schedules for core needle biopsy and resection specimens. Uninhibited growth of tumor is unlikely to be a contributing factor, because resection shortly followed biopsy.

The current study is too recent to generate survival data. However, a recent retrospective study of 176 patients used broad categories of reduction in tumor cellularity and demonstrated a significant correlation with overall and disease free survival at 5 years.[14] In that study, the authors analyzed the histologic response as an independent variable and did not compare this with clinical response or residual pathologic tumor size.[14] That study adds support to our finding that the change in cellularity within the tumor is an independent variable to be included in the pathologic assessment and to be combined with change in the tumor size.

When pathologic CRs (pT0) were excluded, the distribution of pathologic size in treated and control tumors appeared to have similar shapes, distributed asymmetrically around a modal peak. That shape of distribution in the treated group may be interpreted to mean that tumors that do not achieve a pathologic CR are not affected much as a population (Fig. 5B). However, the shape of the distribution of the product of tumor size and tumor cellularity demonstrated a marked left shift in the population to form an inversely sloping curve (Fig. 5D). That distribution suggests that many tumors nearly achieve a pathologic CR. Indeed, the variable cellularity of residual tumors appears to organize the pathologic responses when it is included with residual tumor size (Fig. 5D). Other clinical studies have indicated that smaller residual tumors, such as microscopic residual invasive carcinoma, and even tumors that measure < 1 cm in greatest dimension, are associated with improved survival compared with other residual tumors.[6,16,20] The observed reduction in cellularity in pT1a and pT1b tumors helps to explain this. The histogram we observed for the product of tumor size and cellularity may describe a continuous distribution of pathologic responses and may be more accurate than stratification into categories of response based on tumor size alone.

Studies currently are underway to measure the product of tumor size and cellularity of each tumor before treatment to compare with residual pathologic findings after treatment. It even may be possible to develop a mathematical model from these distributions to compare the responses of entire populations of patients who receive different neoadjuvant chemotherapy regimens. We conclude that tumor cellularity qualifies as an informative parameter for inclusion in a schema to quantify response of breast carcinoma to neoadjuvant chemotherapy.

REFERENCES

1. Bonadonna G, Valagussa, Brambilla C, et al. Primary chemotherapy in operable breast cancer: eight-year experience at the Milan Cancer Institute. *J Clin Oncol.* 1998;16: 93–100.
2. Bryant J, Fisher B, Wolmark N, et al. Effect of preoperative chemotherapy on the outcome of women with operable breast cancer. *J Clin Oncol.* 1998;16:2672–2685.
3. Ferriere JP, Assier I, Cure H, et al. Primary chemotherapy in breast cancer: correlation between tumor response and patient outcome. *Am J Clin Oncol.* 1998;21:117–120.
4. Honkoop AH, van Diest PJ, de Jong JS, et al. Prognostic role of clinical, pathological and biological characteristics in patients with locally advanced breast cancer. *Br J Cancer.* 1998;77:621–626.
5. Lenert JT, Vlastos G, Mirza NQ, et al. Primary tumor response to induction chemotherapy as a predictor of histological status of axillary nodes in operable breast cancer patients. *Ann Surg Oncol.* 1999;6:762–767.
6. Kuerer HM, Newman LA, Smith TL, et al. Clinical course of breast cancer patients with complete pathologic primary and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *J Clin Oncol.* 1999;17: 460–467.
7. Green MC, Buzdar AU, Smith T, et al. Weekly paclitaxel followed by FAC as primary systemic chemotherapy of operable breast cancer improves pathologic complete remission (pCR) rates when compared to every 3-week therapy followed by FAC-final results of a prospective Phase III randomized trial. *Proc Am Soc Clin Oncol.* 2002;21:135.
8. Chollet P, Amat S, Cure H, et al. Prognostic significance of a complete pathological response after induction chemotherapy in operable breast cancer. *Br J Cancer.* 2002;86:1041–1046.
9. Kuerer HM, Newman LA, Buzdar AU, et al. Pathologic tumor response in the breast following neoadjuvant chemotherapy predicts axillary lymph node status. *Cancer J Sci Am.* 1998; 4:230–236.
10. Valero V, Buzdar AU, McNeese M, Singletary E, Hortobagyi GN. Primary chemotherapy in the treatment of breast cancer: the University of Texas M. D. Anderson Cancer Center experience. *Clin Breast Cancer.* 2002;3(Suppl 2):S63–S68.
11. Wolmark N, Wang J, Mamounas E, Bryant J, Fisher B. Preoperative chemotherapy in patients with operable breast cancer: nine-year results from the National Surgical Adjuvant Breast and Bowel Project. *J Natl Cancer Inst Monogr.* 2001;30:96–102.
12. Machiavelli MR, Romero AO, Perez JE, et al. Prognostic significance of pathological response of primary tumor and metastatic axillary lymph nodes after neoadjuvant chemotherapy for locally advanced breast carcinoma. *Cancer J Sci Am.* 1998;4:125–131.
13. Raymond AK, Chawla SP, Carrasco H, et al. Osteosarcoma Chemotherapy effect: a prognostic factor. *Semin Diagn Pathol.* 1987;4:212–236.
14. Ogston KN, Miller ID, Payne S, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast.* 2003;12:320–327.
15. Singletary SE, Allred C, Ashley P, et al. Revision of the American Joint Committee on Cancer staging system for breast cancer. *J Clin Oncol.* 2002;20:3576–3577.

16. Fisher ER, Wang J, Bryant J, Fisher B, Mamounas E, Wolmark N. Pathobiology of preoperative chemotherapy. Findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-18. *Cancer.* 2002;95:681–695.
17. Furukawa Y, Kimijima II, Abe R. Immunohistochemical image analysis of estrogen and progesterone receptors in breast cancer. *Breast Cancer.* 1998;5:375–380.
18. Auger M, Katz RL, Johnston DA, Sneige N, Ordonez NG, Fritsche H. Quantitation of immunocytochemical estrogen and progesterone receptor content in fine needle aspirates of breast carcinoma using the SAMBA 4000 image analysis system. *Anal Quant Cytol Histol.* 1993;15:274–280.
19. Morris EA, Liberman L, Trevisan SG, Abramson AF, Dershaw DD. Histologic heterogeneity of masses at percutaneous breast biopsy. *Breast J.* 2002;8:187–191.
20. Mauriac L, MacGrogan G, Avril A, et al. Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: a unicentre randomized trial with a 124-month median follow-up. Institut Bergonie Bordeaux Groupe Sein (IBBGS). *Ann Oncol.* 1999;10:47–52.